

FIGURE 6A

Numbering is according to Kimura et al.

1. 'A' Allele, CYP2D6*3, A2637 deletion, Frameshift resulting in zero enzyme activity

		2637	
5'-GCTAACCTGAGCAGGATGAC C-3' NH2	CYPwt(+)/A2624/22mer, 54%GC, Tm=63-64C	(SEQ ID NO.))
5'-GCTAACCTGAGCAGGATGAC C (A)30-3' NH2	CYPwt(+)/A2624(A)30-3'NH2	(SEQ ID NO.))
5'-CTAACCTGAGCAGGATGAC C (A)30-3' NH2	CYPwt(+)/A2625(A)30-3'NH2	(SEQ ID NO.))
5'-CTAACCTGAGCAGGATGAC C (A)30-3' NH2	CYPwt(+)/A2625b(A)30-3'NH2	(SEQ ID NO.))
5'-GCTAACCTGAGCAGGATGAC C (A)30-3' NH2	CYPmut(+)/A2625c(A)30-3'NH2	(SEQ ID NO.))
5'-GCTAACCTGAGCAGC - GGATGAC C-3' NH2	CYPmut(+)/A2624/21mer, 57%GC, Tm=61-63C	(SEQ ID NO.))
5'-CTAACCTGAGCAGC - GGATGAC C (A)30-3' NH2	CYPmut(+)/A2625(A)30-3'NH2	(SEQ ID NO.))
5'-CTAACCTGAGCAGC - GGATGAC C (A)30-3' NH2	CYPmut(+)/A2625b(A)30-3'NH2	(SEQ ID NO.))
5'-CTAACCTGAGCAGC - GGATGAC C (A)30-3' NH2	CYPmut(+)/A2625c(A)30-3'NH2	(SEQ ID NO.))
5'-gctaacctgagcaggatg (A)30-3' NH2	CYPwt(+)/A2624b(A)30-3'NH2	(SEQ ID NO.))
NH2 3'-(A)30 g a t t g a c t c g t g t c c t a c t g -5'	CYPwt(-)/A2625(A)30-3'NH2	(SEQ ID NO.))
x= 2-Amino-dA	5'-c t g x g c a c x g g a t g x c (A)30-3' NH2	CYPwt(+)/2629a(A)30-3'NH2	(SEQ ID NO.)
x= C-5 propynyl-C	5'-x t g a g x a c a g g a t g a x (A)30-3' NH2	CYPwt(+)/2629b(A)30-3'NH2	(SEQ ID NO.)
x= C-5 propynyl-C	5'-x t g a g c a x a g g a t g a x (A)30-3' NH2	CYPwt(+)/2629c(A)30-3'NH2	(SEQ ID NO.)
5'-GCTGGGATGAGCTGCTAACCTGAGCAGGATGACCTGGGAC C C A G C C C A G C C-3'	Wild Type (+)	(SEQ ID NO.))
5'-GCTGGGATGAGCTGCTAACCTGAGCAGC - GGATGACCTGGGAC C C A G C C C A G C C-3'	Mut (+)	(SEQ ID NO.))

FIGURE 6B

		1934			
		NH2 3'- G A G G G T G G G G T C C T G C -5'		CYPwt(-)B1922, 17mer, 76%GC, Tm=66C	
		5'- C T C C A C C C C A G A C G -3' NH2		CYPwt(+)B1922, Target	
		5'- C T C C A C C C C A A G A C G -3' NH2		CYPwt(+)B1922, 71%GC, Tm=58-60C	
		NH2 3'- G A G G T G G G G T C T G C -5'		CYPmut(-)B1922-Target	
		5'- C C C T A C C C G C A T C T C C C A C C C C A A G A C G C C C C T T C G C C C A A C G G T C T -3'		Wild Type (+)	
		5'- C C C T A C C C G C A T C T C C C A C C C C A A G A C G C C C C T T C G C C C A A C G G T C T -3'		Mut (+)	
		B. CYPwt(-)B1930 (C/A to mut at base 13) and			
		CYPmut(+)B1930 (A/C to wt at base 5)			
		NH2 3'- G G G T C C T G C G G G A A A G -5'		CYPwt(-)B1930, 17mer, 71%GC, Tm=56C	
		NH2 3'-(A)30 G G G T C C T G C G G G A A A G -5'		CYPwt(-)B1930(A)30-3'NH2	
		5'- C C C A A G A C G C C C T T T C -3' NH2		CYPmut(+)B1930, 17mer, 65%GC, Tm=54C	
		5'- C C C A A G A C G C C C T T T C (A)30-3' NH2		CYPmut(+)B1930(A)30-3'NH2	
		5'- C C C T T A C C C G C A T C T C C A C C C C C A A G A C G C C C T T T C G C C C A A C G G T C T -3'		Wild Type (+)	
		5'- C C C T T A C C C G C A T C T C C A C C C C C A A G A C G C C C C T T T C G C C C A A C G G T C T -3'		Mut (+)	
		3. 'C' Allele, CYP2D6*9, G2702-A2704 deletion, decreased enzyme activity			
		-2702			
		5'- G C A G A G A T G G A G A A G G T G A G A G -3' NH2		CYPwt(+)C2691, 22mer, 55%GC, Tm=60C	
		5'- G C A G A G A T G G A G A A G G T G A G A G (A)30-3' NH2		CYPwt(+)C2691(A)30-3'NH2	
		5'- C A G A G A T G G A G A G G T G A G A G (A)30-3' NH2		CYPwt(+)C2692(A)30-3'NH2	
		5'- G C A G A G A T G G A - - - G G T G A G A G T G -3' NH2		CYPmut(+)C2691, 21mer, 57%GC, Tm=60C	
		5'- G C A G A G A T G G A - - - G G T G A G A G T G (A)30-3' NH2		CYPmut(+)C2691(A)30-3'NH2	
		5'- C A G A G A T G G A - - - G G T G A G A G T G (A)30-3' NH2		CYPmut(+)C2692(A)30-3'NH2	
		3'- T G A C T C C G G A A G G A C C G T C T A C C T - - - C C A C T C T C A C C G A C G G T G C C A C -5'		Wild Type (-)	
		3'- T G A C T C C G G A A G G A C C G T C T A C C T - - - C C A C T C T C A C C G A C G G T G C C A C -5'		Mut (-)	

FIGURE 6C

4. 'E' Allele, CYP2D6*7, A3023C, H324P amino acid change results in zero enzyme activity

A. wt Probe - CYPwt(-)E3009 (T/C to mut at base 5) & CYPmut(+E3009 (C/A to wt at base 15)

		3023	
NH2 3'-(A)30	C G A G T A C T A G G A T G T A G G C -5'	CYPwt(-)E3009, 19mer, 53%GC, Pred Tm=57	(SEQ ID NO.)
5'-G C T C A T G A T C C T A C C T C C G -3' NH2		CYPwt(-)E3009(A)30-3'NH2	(SEQ ID NO.)
5'-G C T C A T G A T C C T A C C T C C G (A)30-3' NH2		CYPmut(+E3009, 19mer, 58%GC, Pred Tm=59C	(SEQ ID NO.)
5'-T G G G G C C T C C T G C T C A T G A T C C T A C C A T C C G G A T G T G C A G C		CYPmut(+E3009(A)30-3'NH2	(SEQ ID NO.)
5'-T G G G G C C T C C T G C T C A T G A T C C T A C C T C C G G A T G T G C A G C		G T G A G C C C A T C -3' Wild Type (+)	(SEQ ID NO.)
		G T G A G C C C A T C -3' Mut (+)	(SEQ ID NO.)

B. CYPwt(-)E3018 (T/C to mut at base 14) and

		CYPmut(+E3018 (C/T to wt at base 6)	
NH2 3'-G G A T G T A G G C C T A C A C G T C -5'		CYPwt(-)E3018, 19mer, 58%GC, Tm=60	(SEQ ID NO.)
5'-C C T A C A T C C G G A T G T G C A G -3'		CYPwt(+E3018- Target	(SEQ ID NO.)
5'-C C T A C C T C C G G A T G T G C A G -3' NH2		CYPmut(+E3018, 19mer, 63%GC, Tm=62C	(SEQ ID NO.)
3'-G G A T G G A G G C C T A C A C G T C -5'		CYPmut(-)E3018- Target	(SEQ ID NO.)
5'-T G G G G C C T C C T G C T C A T G A T C C T A C A T C C G G A T G T G C A G C		G T G A G C C C A T C -3' Wild Type (+)	(SEQ ID NO.)
5'-T G G G G C C T C C T G C T C A T G A T C C T A C C T C C G G A T G T G C A G C		G T G A G C C C A T C -3' Mut (+)	(SEQ ID NO.)
		-3038-Intron Start	(SEQ ID NO.)

5. 'G' Allele, CYP2D6*8, G1846T, Stop codon, zero enzyme activity

		1846	
NH2 3'-(A)30	G T G A G G C C A C C C A C T A C C -5'	CYPwt(+G1840(A)30-3'NH2, 18mer, 67%GC, Tm=60	(SEQ ID NO.)
5'-C A C T C C T G T G G G T G A T G G (A)30-3' NH2		CYPwt(-)G1840(A)30-3'NH2	(SEQ ID NO.)
5'-G T G C C G C C T T C G C C A C T C C T G T G G G T G A T G G G C A G A A G G G C A C A A A G C G G -3'		CYPmut(+G1840(A)30-3'NH2, 18mer, 61%GC, Tm=57	(SEQ ID NO.)
5'-G T G C C G C C T T C G C C A C T C C T G T G G G T G A T G G G C A G A A G G G C A C A A A G C G G -3'			(SEQ ID NO.)
		Exon 3 end-1846	(SEQ ID NO.)

FIGURE 6D

6. T' Allele, CYP2D6*6, T1795 deletion, Frameshift resulting in zero enzyme activity

5'-G C T G G A G C A G T G G G T G A C -3' NH2	CYPwt(+)/T1785, 18mer, 67%GC, Tm=59-61C	(SEQ ID NO.)
5'-G C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)/T1785(A)30-3'NH2	(SEQ ID NO.)
5'-C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)/T1786(A)30-3'NH2	(SEQ ID NO.)
5'-G C T G G A G C A G - G G G T G A C -3' NH2	CYPmut(+)/T1785, 17mer, 71%GC, Tm=58-60C	(SEQ ID NO.)
5'-G C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)/T1785(A)30-3'NH2	(SEQ ID NO.)
5'-C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)/T1786(A)30-3'NH2	(SEQ ID NO.)
5'-G G G C A A G A A G T C G C T G G A G C A G T G G G T G A C C G A G G A G G C C G C C T G C C T -3'	Wild Type (+)	(SEQ ID NO.)
5'-G G G C A A G A A G T C G C T G G A G C A G - G G G T G A C C G A G G A G G C C G C C T G C C T -3'	Mut (+)	(SEQ ID NO.)

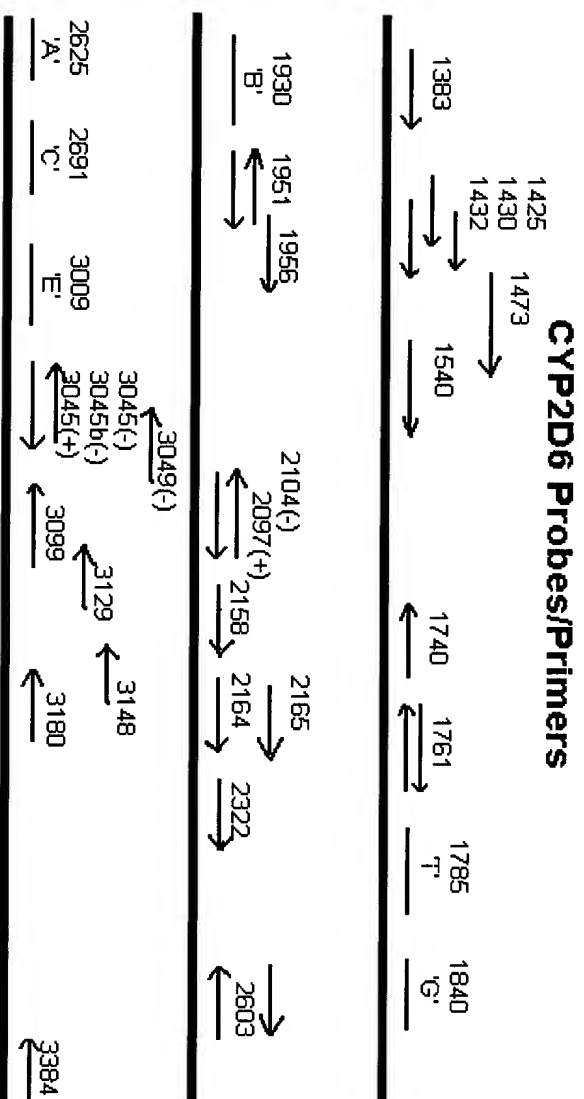
7. 2D6/2D7/2D8 Controls - The 2D6/7/8 probes were designed in the 1600 region of the 2D6 gene. The purpose of the designs was to find region somewhere between the PCR primers were it would be easy to discriminate between 2D6 and its two pseudogenes, 2D7 and 2D8. The purpose of the designs is to demonstrate that the PCR amplicon is from the 2D6 gene, not one of the pseudogenes.

5'-G A C C A G G G G A G C - A T A G G (A)30-3' NH2	CYP2D6wt(+)/1607(A)30-3'NH2	(SEQ ID NO.)
5'-G A C C T T G T G A G C C C A G (A)30-3' NH2	CYP2D7wt(+)/1607(A)30-3'NH2	(SEQ ID NO.)
5'-G A C C A G G A A A A G C - A C A G G (A)30-3' NH2	CYP2D8wt(+)/1607(A)30-3'NH2	(SEQ ID NO.)
5'-G A C C A G G A A A A G C - A C A G G (A)30-3' NH2	CYP2D8wt(+)/1607b(A)30-3'NH2	(SEQ ID NO.)
5'-G G G A G A C C A G G G G A G C - A T A G G G T T G G A G T G G G T G G T -3' 2D6 (+)		(SEQ ID NO.)
5'-G G G A G A C C T T G T G A G C C C A G G G T T G G A G T G G G T G G C -3' 2D7 (+)		(SEQ ID NO.)
5'-G G G A G A C C A G G A A A A G C - A C A G G G T T G G A G T G G G C G G C -3' 2D8 (+)		(SEQ ID NO.)

8. Pos/Neg Control probes- These probes were designed as true positive and negative control probes. They consist of the same semi-random sequence, with the positive control probe having a 5' Biotin.

5' Biotin- A T C A T T C C A A T C A T C C A T A T C A T C (A)25-3' NH2	CYP(+)/an(A)25-5Biotin,3'NH2	(SEQ ID NO.)
5'- A T C A T T C C A A T C A T C C A T A T C A T C (A)25-3' NH2	CYP(+)/an(A)25-3'NH2	(SEQ ID NO.)

Figure 7



CYPwt(+1383, CYPwt(+2097, CYPwt(-2104, and CYPwt(-3180 are published primer sequences.

1. Chen et al., Clinical Pharmacology and Therapeutics, Vol 60, 5:522-34

2. Heim M, Meyer UA. Lancet 1990; 336:529-32

CYPwt(+1540 and CYPwt(-3099 are primers obtained from Intel, referred to as MP3 and MP4 respectively.